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Research Report

# Alterations of the emotional processing system may underlie preserved rapid reaction time in tinnitus

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ABSTRACT

Although alterations of the limbic system have been linked to tinnitus persistence, the neural networks underlying such alteration are unclear. The present study investigated the effect of tinnitus on emotional processing in middle-aged adults using functional magnetic resonance imaging and stimuli from the International Affective Digital Sounds database. There were three groups of participants: bilateral hearing loss with tinnitus (TIN), age- and gender-matched controls with bilateral hearing loss without tinnitus (HL) and matched normal hearing controls without tinnitus (NH). In the scanner, subjects rated sounds as pleasant, unpleasant, or neutral. The TIN and NH groups, but not the HL group, responded faster to affective sounds compared to neutral sounds. The TIN group had elevated response in bilateral parahippocampus and right insula compared to the NH group, and left parahippocampus compared to HL controls for pleasant relative to neutral sounds. A region-of-interest analysis detected increased activation for NH controls in the right amygdala when responding to affective stimuli, but failed to find a similar heightened response in the TIN and HL groups. All three groups showed increased response in auditory cortices for the affective relative to neutral sounds comparisons. Our results suggest that the emotional processing network is altered in tinnitus to rely on the parahippocampus and insula, rather than the amygdala, and this alteration may maintain a select advantage for the rapid processing of affective stimuli despite the hearing loss. The complex interaction of tinnitus and the limbic system should be accounted for in development of new tinnitus management strategies.

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## 1. Introduction

Subjective tinnitus, often described as ringing in the ears, is a common auditory disorder and is frequently associated with hearing loss (Adams et al., 1999; Lockwood et al., 1998). Tinnitus has been noted in patients with severed auditory nerves, which suggests that the internal noise need not originate in the periphery but may have correlates in the central nervous system (House and Brackmann, 1981; Lockwood et al., 2002). Behavioral studies were among the first to show an association between tinnitus and emotional processing exemplified in impaired sleeping habits, increased stress level, frustration, depression, irritability, and anxiety (Andersson and Vretblad, 2000; Andersson, 2002; Bartels et al., 2010; Hallam et al., 1988; Rutter and Stein, 1999; Sindhusake et al., 2004; Tyler and Baker, 1983). In recent years, studies have utilized neural imaging techniques to advance our knowledge of the neural correlates of tinnitus (Burton et al., 2012; Golm et al., 2013; Haller et al., 2010; Leaver et al., 2011; Mirz et al., 2000b). However, few studies have explicitly investigated the limbic-auditory link using functional brain imaging tools. A reason tinnitus remains difficult to treat is because mechanisms for its persistence are not fully understood. Therefore, the goal of the present study was to investigate the neural alterations associated with tinnitus, related to changes in the limbic system reflected in emotional processing of unpleasant and pleasant sounds.

Alterations of the limbic system associated with bothersome tinnitus is one of the most studied mechanisms in tinnitus persistence (Henry et al., 2002; Jastreboff, 1990; Jastreboff and Jastreboff, 2000; Mirz et al., 2000a; Muhlau et al., 2006; Shargorodsky et al., 2010). Jastreboff (1990) advanced the idea that the amygdala, a key region of the limbic system, plays a central role in the development of bothersome tinnitus. He suggested that the tinnitus signal is similar to an alarm bell, which can have a range of emotional associations. The negative emotional significance given to the signal from the limbic system enhances its detection and results in the tinnitus percept becoming chronic. Jastreboff's theory is supported by recent findings of Kumar et al. (2012). Kumar and his colleagues collected fMRI data from normal hearing adults and employed effective connectivity analysis to evaluate connections between the auditory cortex and the amygdala. They observed that the backward connections from the amygdala to the auditory cortex are associated with the evaluation of emotional content of sounds and the forward connections are associated with the evaluation of acoustic information (Kumar et al., 2012). The feedback connections from the amygdala to the auditory cortex may explain the emotional significance attributed to the tinnitus precept. Therefore one hypothesis, tested in the present study, was that individuals with tinnitus would show a heightened response in the amygdala to affective sounds, in particular to the unpleasant sounds.

In addition to the amygdala, other limbic regions associated with tinnitus are parahippocampus and insula. De Ridder et al. (2011) conducted a resting state EEG study on individuals with mild to severe tinnitus and found that tinnitus-related distress is correlated with abnormal activity in a network consisting of frontal and limbic structures, including the parahippocampus and insula. An fMRI study, which involved reading unpleasant and neutral sentences, noted increased activity in the insula and frontal regions in individuals with tinnitus compared to non-tinnitus controls while viewing unpleasant sentences (Golm et al., 2013). The authors proposed that the increased activity in the insula may be correlated with tinnitus distress. Note that the Golm et al. (2013) group used unpleasant stimuli and they did not find significant differences in parahippocampal response between groups. Based on these studies, we expected that individuals with tinnitus would show a heightened response in the parahippocampus and the insula to affective sounds, and this would be most notable for the unpleasant sounds.

Previous studies have also implicated the ventral medial prefrontal cortex (vmPFC) and nucleus accumbens (NAc) as regions associated with tinnitus. Structural imaging studies have provided evidence that the vmPFC and NAc may typically work toward the cessation and decreased intrusiveness of tinnitus by stimulating the thalamic reticular nucleus intercepting of the tinnitus signal and preventing its conscious perception (Muhlau et al., 2006; Rauschecker et al., 2010). Therefore decreased functioning of the vmPFC and NAc may be associated with tinnitus persistence.

In addition to limbic regions, elevated response patterns have also been observed in central auditory areas in tinnitus. Major regions shown to exhibit hyperactivity in tinnitus are the inferior colliculus and auditory cortex (Jastreboff and Sasaki, 1986; Leaver et al., 2011; Melcher et al., 2009; Zhang et al., 2011). Elevated response from the inferior colliculus in subjects with tinnitus has been shown in human studies using fMRI and broadband band sound presented to tinnitus and non-tinnitus controls (Melcher et al., 2009). Additionally, in a study using fMRI and sounds matched to the subject's tinnitus, hyperactivity in the auditory cortex was observed in tinnitus (Leaver et al., 2011). Therefore, we anticipated the tinnitus group would exhibit increased response in auditory regions. Based the aforementioned impact of tinnitus on auditory regions and the auditory nature of the disorder, we used sounds instead of non-auditory stimuli because of a greater expectation of the impact of tinnitus on the auditory modality.

The international affective digital sounds (Bradley and Lang, 2007), used in the present study, were previously rated by young, healthy participants to be pleasant, unpleasant, and neutral. The few studies that have investigated tinnitus using functional imaging and affective stimuli have primarily used unpleasant or neutral stimuli (Golm et al., 2013; Mirz et al., 2000b; Schlee et al., 2008). For instance, the Golm et al. (2013) group displayed unpleasant and neutral sentences to the volunteers. Additionally, a recent functional connectivity study used unpleasant tones matched to an individual's tinnitus and neutral tones different than the individual's tinnitus to evoke a response from the tinnitus network (Schlee et al., 2008). Confirming their prediction, they found the unpleasant tones stimulated the tinnitus network to a greater extent than the control sounds (Schlee et al., 2008). The Mirz et al. (2000a) research team used an unpleasant tinnitus-like tone to investigate how tinnitus may alter brain function in healthy adults. As expected, they found increased

activation of limbic regions including the amygdala, parahippocampus and insula for the unpleasant tinnitus-like tones in healthy volunteers (Mirz et al., 2000b). Given the impact of aversive stimuli we predicted that the unpleasant sounds would illustrate emotional processing differences between the tinnitus and control groups. However, because pleasant sounds have not been used previously to investigate the neural bases of tinnitus, they were included in the present study. We expected pleasant sounds to be useful in differentiating between tinnitus and non-tinnitus groups.

The present study addresses a gap in knowledge surrounding functional brain measurements concerning alterations in emotional processing associated with tinnitus. Functional imaging studies have implicated the emotional processing system as a set of large-scale neural network engaged in tinnitus, but few have directly tested it. Studies that have used fMRI and emotionally salient stimuli to investigate tinnitus have primarily focused on individuals with severe tinnitus (Golm et al., 2013; Schlee et al., 2008), simulated tinnitus in a non-tinnitus population (Mirz et al., 2000b), or employed non-auditory stimuli (Golm et al., 2013). Additionally, the contributions of hearing loss has not been controlled for by including a hearing loss without tinnitus control group (De Ridder et al., 2011; Golm et al., 2013; Mirz et al., 2000b; Muhlau et al., 2006; Rauschecker et al., 2010; Schlee et al., 2008). To advance the knowledge of alterations in the emotional processing system associated with tinnitus, the current investigation used both pleasant and unpleasant stimuli, and controlled for the influence of hearing loss.

The present study implemented an affective sound rating task to estimate behavioral and neural differences in the engagement of the emotional processing network in individuals with hearing loss and tinnitus and their control groups without tinnitus, one with hearing loss and the other with normal hearing. We tested the hypothesis that amygdalar response would be elevated in the tinnitus group compared to controls. Increased response from the parahippocampus and insula was also expected in the tinnitus group compared to controls. Furthermore, we tested the hypothesis that the tinnitus group would show heightened response in auditory regions relative to the normal hearing and hearing loss groups. Lastly, we expected both the pleasant and unpleasant sounds to be useful in differentiating between tinnitus and non-tinnitus groups; however, we anticipated the unpleasant sounds would parse group differences to a larger extent.

## 2. Results

Three groups were recruited for the study, as follows: bilateral hearing loss with tinnitus (TIN), bilateral hearing loss without tinnitus (TIN), and normal hearing without tinnitus (NH). Subjects rated 90 affective sounds (30 pleasant, 30 unpleasant, 30 neutral) using button presses, while in the MRI scanner. The following is a brief summary our statistical methods. First level fixed effects analysis was performed on each subject to generate P>N and U>N contrast images (or conditions) for the flexible group-level factorial model, in which all the following analyses were conducted. Whole-brain voxelwise analysis was implemented to compute main effect of group, main effect of condition and interaction between group and condition. Between group whole-brain *t*-tests were conducted to better understand the directionality of the *F*-test results. Based on our *a prior* hypothesis concerning the involvement of auditory and limbic regions in tinnitus, we directly compared groups using post-hoc two-sample *t*-tests with ROI voxelwise analysis. Within group whole-brain analysis was also conducted.

### 2.1. *Behavioral results*

#### 2.1.1. *Faster response times to emotional sounds for both TIN and NH groups, but not in HL group*

A two-way ANOVA test was conducted using reaction time as the dependent variable with group and condition as independent factors. There was a statistically significant main effect of group ($p<0.000001$), a main effect of condition ($p<0.000001$), and an interaction between group and condition ($p<0.001$) for the reaction time data. Fig. 2 illustrates that

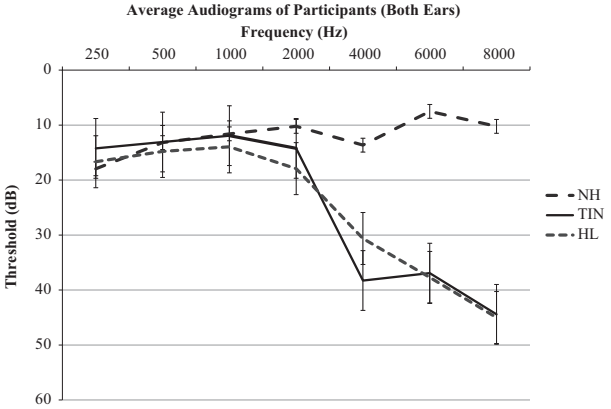


**Fig. 1 – Average audiograms (combined values of both ears) of fMRI subjects including error bars that depict standard error of the mean.**

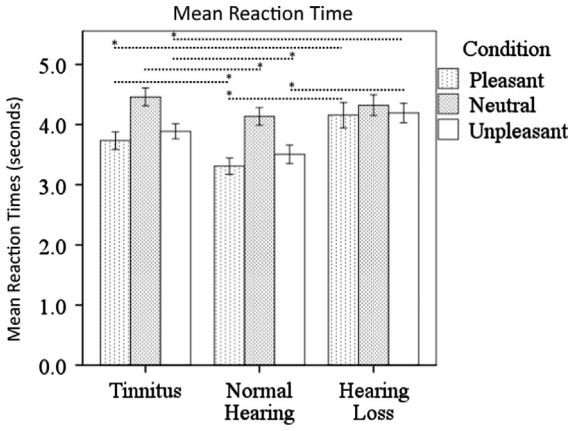


**Fig. 2 – *Task based reaction time results.* TIN and NH reaction times were significantly slower for neutral sounds compared to pleasant and unpleasant sounds. The TIN group responded slower to the pleasant, unpleasant and neutral sounds compared to the NH group. The HL group responded slowest to the pleasant and unpleasant sounds. Statistical significance level $p<0.05$ indicated by *.**

the TIN group maintained an analogous pattern as the NH group, and responded significantly slower to the neutral sounds compared to the P and U sounds (Fig. 2). The HL group differed from the other two groups with the reaction times being similar for all three types of sounds (Fig. 2). When comparing across groups, the HL group was significantly slower, for both P and U sounds, compared to the NH ($p<0.000001$; $p<0.000001$) and TIN ($p<0.0003$; $p<0.00009$) groups (Fig. 2). Although it did not reach statistical significance, Spearman correlational analysis revealed a minor trend for increased amygdala activation to be correlated with faster reaction times (Supplementary Fig. 1).

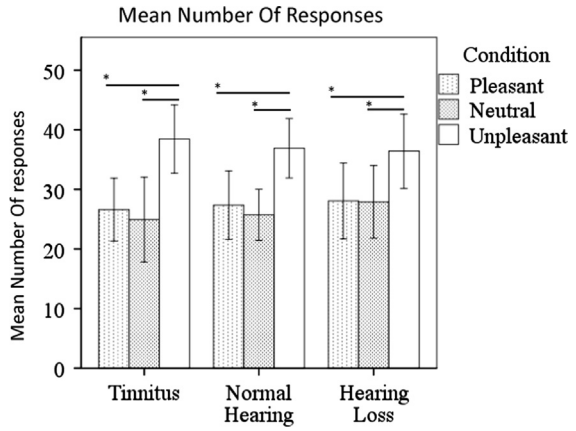


**Fig. 3 – *Task based rating results.* All three groups rated sounds as unpleasant significantly more than neutral or pleasant. Statistical significance level $p<0.05$ indicated by *.**

A two-way ANOVA test was conducted in the same manner as previously discussed for the ratings of sounds. There was a significant main effect of condition ($p<0.00001$) in the ratings of the sounds as belonging to P, U, or N categories. All three groups rated significantly more sounds as U compared to P or N; Fig. 3 illustrates this similarity. Note that an equal number of sounds classified as P, N, and U, according to the published normative IADS scores, were included in the experiment (Bradley and Lang, 2007).

### 2.2. *fMRI results*

Within the flexible factorial model, main effect of group, main effect of condition, and interaction between condition and group factors were computed using whole-brain voxel-wise analysis. For the main effect of group, differences in neural response were observed in bilateral precuneus, bilateral middle temporal gyrus, bilateral inferior parietal lobule, and the left middle frontal gyrus for pleasant and unpleasant sounds relative to neutral (Table 2). For the main effect of condition, differences in neural response were localized in medial frontal gyrus and the anterior cingulate (Table 2). Interaction between group and condition did not reach statistical significance at the threshold involved in the present analyses.

**Table 1 – Subject demographics and clinical characteristics for all three groups.**

| Group: | NH (normal hearing) | TIN (tinnitus) | HL (hearing loss) |
|---|---|---|---|
| Group size | 12 | 13 | 12 |
| Age (M±SD) | 51.4±9.9; 41–64 | 54.7±7.0; 42–64 | 58.2±9.5; 39–71 |
| Gender | 6 Male, 6 female | 9 Male, 4 female | 5 Male, 7 female |
| BAI (M±SD) | 1.25±1.3; 0–4 | 1.7±1.6; 0–3 | 2.3±1.7; 0–6 |
| BDI-II (M±SD) | 1.7±2.3; 0–8 | 1.3±1.9; 0–6 | 4.3±4.1; 0–10 |
| THI (M±SD) | – | 8.3±6.8; 0–18 | – |

BDI=Beck depression inventory, BAI=Beck anxiety inventory, THI=tinnitus handicap inventory.

**Table 2 – Local maxima for the main effect of group and condition.**

| Contrast | MNI coordinates X, Y, Z | Z score | Cluster ($mm^3$) | Brain region (Brodmann area) |
|---|---|---|---|---|
| Main effect group | −18, −58, 42 | 5.88 | 627 | L. precuneus (BA 7) |
| | 12, −66,44 | 5.64 | | R. precuneus (BA 7) |
| | 46, −62,22 | 5.83 | 103 | R. middle temporal gyrus (BA 39) |
| | 36, −58, 42 | 5.10 | | R. inferior parietal lobule (BA 40) |
| | −44, −36,26 | 5.32 | 354 | L. inferior parietal lobule (BA 40) |
| | −36,42,20 | 5.15 | 402 | L. middle frontal gyrus (BA 46) |
| | −34, −68,20 | 4.90 | 310 | L. middle temporal gyrus (BA 39) |
| Main effect condition | 6, 54, 4 | 5.38 | 1728 | Medial frontal gyrus (BA 10) |
| | −4,52,2 | 5.30 | | L. anterior cingulate (BA 10) |
| | −4,38,4 | 5.08 | | L. anterior cingulate (BA 24) |

Reported regions are listed in Montreal Neurological Institute (MNI) coordinates and in terms of Brodmann areas (before determining the Brodmann areas, the MNI coordinates were converted to Talairach coordinates). Statistical threshold was set at $p<0.05$ FWE corrected for multiple comparisons. L, left; R, right.

**Table 3 – Local maxima for whole-brain analysis for inter-group contrasts.**

| Contrast | MNI coordinates X, Y, Z | Z score | Cluster | Gyrus (Brodmann area) |
|---|---|---|---|---|
| TIN > NH (P > N) | −26, −68,42 | 5.23 | 354 | L. precuneus (BA 19) |
| | 4, −22,26 | 5.04 | 183 | Corpus callosum |
| | −32, 16, 26 | 4.90 | 257 | L. middle frontal gyrus (BA 46) |
| | −36,22,20 | 4.83 | | L. inferior frontal gyrus (BA 45) |
| | 24, −20, −12 | 4.79 | 83 | R. parahippocampal |
| | 18, −62,44 | 4.77 | 104 | R. precuneus (BA 7) |
| TIN > NH (U > N) | −26, −68,42 | 5.08 | 185 | L. precuneus (BA 19) |
| NH > TIN (P > N) | | | | No suprathreshold voxels |
| NH > TIN (U > N) | | | | No suprathreshold voxels |
| TIN > HL (P > N) | 8, −60,28 | 4.76 | 261 | Posterior cingulate gyrus (BA 31) |
| TIN > HL (U > N) | | | | No suprathreshold voxels |
| HL > TIN (P > N) | | | | No suprathreshold voxels |
| HL > TIN (U > N) | | | | No suprathreshold voxels |
| NH > HL (P > N) | 44, −60,22 | 5.06 | 88 | R. middle temporal gyrus(BA 39) |
| NH > HL (U > N) | 42,30,18 | 5.20 | 249 | R. middle frontal gyrus (BA 46) |
| | −14,34,26 | 4.98 | 229 | L. anterior cingulate (BA 32) |
| HL > NH (P > N) | 16, −64,44 | 5.13 | 311 | R. precuneus (BA 7) |
| | −18, −58,40 | 5.01 | 155 | L. precuneus (BA 7) |
| HL > NH (U > N) | | | | No suprathreshold voxels |

Whole-brain two sample *t*-test were computed to identify between group differences. Reported regions are listed in Montreal Neurological Institute (MNI) coordinates and in terms of Brodmann areas (before determining the Brodmann areas the MNI coordinates were converted to Talairach coordinates). Statistical threshold was set at $p<0.05$ FWE corrected for multiple comparisons. L, left; R, right.

In order to present a complete picture, we investigated the directionality of the above results using between-group whole-brain two sample *t*-tests, within the flexible factorial model (Table 3). Increased response was observed in the left middle frontal gyrus for the TIN > NH (P > N) comparison and elevated response was detected in the right middle temporal gyrus for NH > HL (U > N) (Table 3). Based on our specific *a prior* hypotheses, we focused on subsequent targeted ROI analysis.

#### 2.2.1. *Amygdalar response detected only in the NH group*

To better detect amygdala activation, within group ROI analysis of the amygdala was conducted. Contrary to our hypothesis that individual with tinnitus would show a heightened response in the amygdala, predominantly to unpleasant sounds, increased amygdala response was not obtained for the TIN group, neither in the P > N nor in the U > N contrast (Table 5). Although the amygdala response did not survive correction, it was detected at $p<0.001$ uncorrected in the NH group (for both P > N and U > N contrasts), but not in the TIN or HL groups, as illustrated by Fig. 4 and presented in Table 5. Note that a decreasing trend of NH > HL > TIN was observed for both the P > N and U > N contrasts. However, upon direct comparison between groups with post-hoc two sample *t*-tests using ROI analysis that included amygdala, increased response was not obtained for either condition.

#### 2.2.2. *Heightened parahippocampal and insular response detected for the TIN group compared to controls, when processing pleasant sounds*

An unexpected result was the finding that the pleasant sounds appeared to differentiate the TIN group from the control groups to a greater extent than the unpleasant sounds. Direct comparisons were computed with post-hoc two sample *t*-tests using ROI analysis that included insula and parahippocampus. For the TIN > NH (P > N) comparison, heightened response in bilateral parahippocampus and right insula was observed (Fig. 5; Table 6). Similarly, in the TIN > HL (P > N) contrast elevated response was detected in the left parahippocampus and medial frontal gyrus (Fig. 5; Table 6). There were no suprathreshold voxels for the TIN > NH (U > N) and TIN > HL (U > N) comparisons (Table 6). For a complete list refer to Table 6.

#### 2.2.3. *All three groups exhibited similar response patterns in auditory regions*

The TIN, NH, and HL groups showed increased response in overlapping auditory regions for within group whole-brain voxelwise analysis (Fig. 6). In the NH group, areas of increased activation for the contrast P > N were observed in bilateral superior temporal gyri, right middle temporal gyrus, and right transverse temporal gyrus (Table 4). For the NH (U > N) contrast, elevated response was observed in bilateral superior temporal gyri, bilateral middle temporal gyri, and right transverse temporal gyrus (Table 4). Concerning the HL (P > N) contrast, increased response was obtained in bilateral superior temporal gyri, right transverse temporal gyrus, and right middle temporal gyrus (Table 4). For the HL (U > N) comparison, heightened activation was observed in bilateral superior temporal gyri and left transverse temporal gyrus (Table 4). In the TIN group, elevated response was obtained in bilateral superior temporal gyri, and left transverse temporal gyrus, for the P > N comparison (Table 4). For the U > N comparison, the TIN exhibited increased response in bilateral transverse temporal gyri and right superior temporal gyrus (Table 4). Direct comparisons between groups with two sample *t*-tests using ROI analysis that included inferior

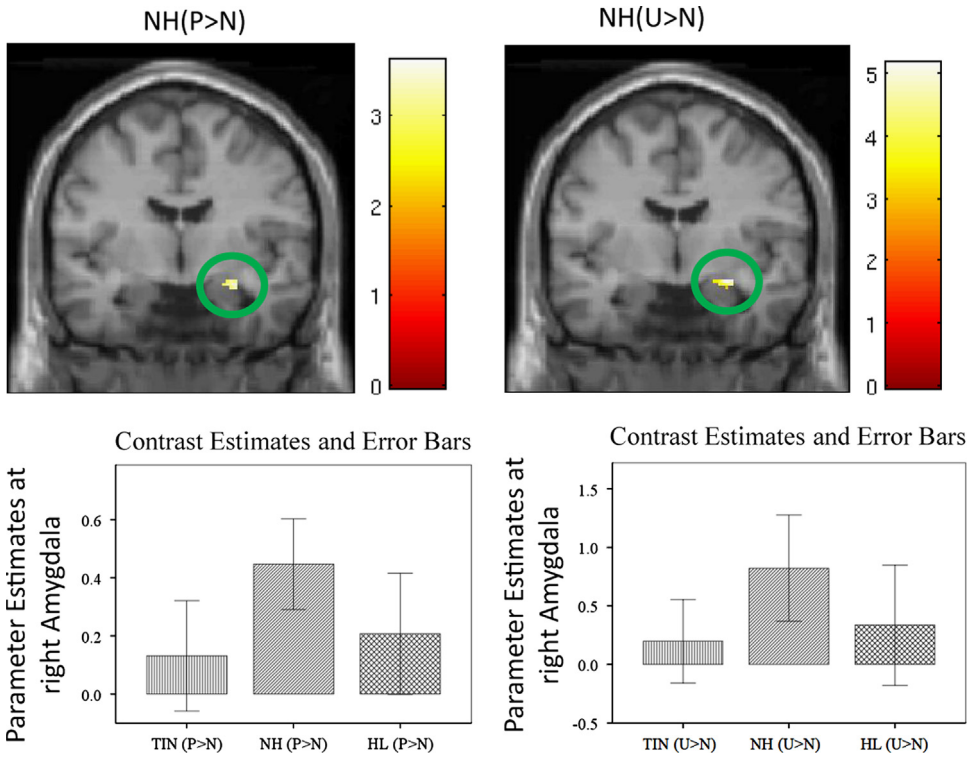


**Fig. 4 – *Statistical parametric maps for amygdala region-of-interest analysis (ROI).* Within group ROI analysis of the amygdala revealed heightened right amygdala response for the P > N and U > N comparison for the NH group at $p < 0.001$ uncorrected. Decreasing amplitude of effect in the right (R) amygdala was obtained in the three groups in this order: NH > HL > TIN, for both the P > N and U > N contrasts. It is important to note that direct comparison with two sample t-tests failed to detect increased amygdala response for either contrast. For illustration purposes, the ROIs for R amygdala are shown at $p < 0.005$ uncorrected, but the clusters in the circles are significant at $p < 0.001$ uncorrected.**

colliculus, medial geniculate body and primary auditory cortex did not detect suprathreshold voxels in the defined auditory regions (Table 6). For a complete list of activated regions, refer to Table 4.

## 3. Discussion

Our study revealed four main findings. First, behaviorally, the TIN group maintained a selective advantage for processing pleasant and unpleasant sounds faster than neutral stimuli, which paralleled the NH group, whereas the HL group did not exhibit this pattern. Second, contrary to our expectations, tinnitus patients did not show greater engagement of the amygdala during affective sound processing relative to controls. Third, compared to controls, the TIN group exhibited an increased response in parahippocampus and insula, for the processing of pleasant compared to neutral sounds. Finally, no differences were observed in auditory regions between the TIN group and the controls upon direct comparison, including in specific targets such as inferior colliculus and medial geniculate body. Therefore, the greatest influence of tinnitus may be outside primary auditory areas, in the limbic regions of parahippocampus and insula. Our results suggest that emotional processing in tinnitus is altered; however, it is changed in a more complex manner than hypothesized and possibly the processing of pleasant sounds is most affected. These findings are discussed in turn below.

### 3.1. *Faster response times to emotional sounds for both TIN and NH groups, but not in HL group*

Past studies have suggested that individuals with tinnitus may need to simultaneously attend to their tinnitus and externally relevant stimuli, which may deplete resources available to complete listening tasks and impair reaction time (Dornhoffer et al., 2006; Hallam et al., 2004; Stevens et al., 2007). In the present study, the TIN group paralleled the reaction time pattern of the NH group (i.e., faster responses to affective sounds compared to neural sounds), but the HL group did not show this pattern. Note that the TIN group did not have faster reaction times than the NH group, rather the two groups, TIN and NH, showed similar reaction time patterns not exhibited by the HL group. It has been shown that stimuli which engage the limbic system, primarily the amygdala, are privileged to rapid, bottom up processing (Shafer et al., 2012; Vuilleumier et al., 2001). In accordance with these findings, the group that had the strongest amygdalar response, normal hearing controls, processed the affective stimuli the fastest as indicated by their reaction time data. Interestingly, improved reaction time to affective

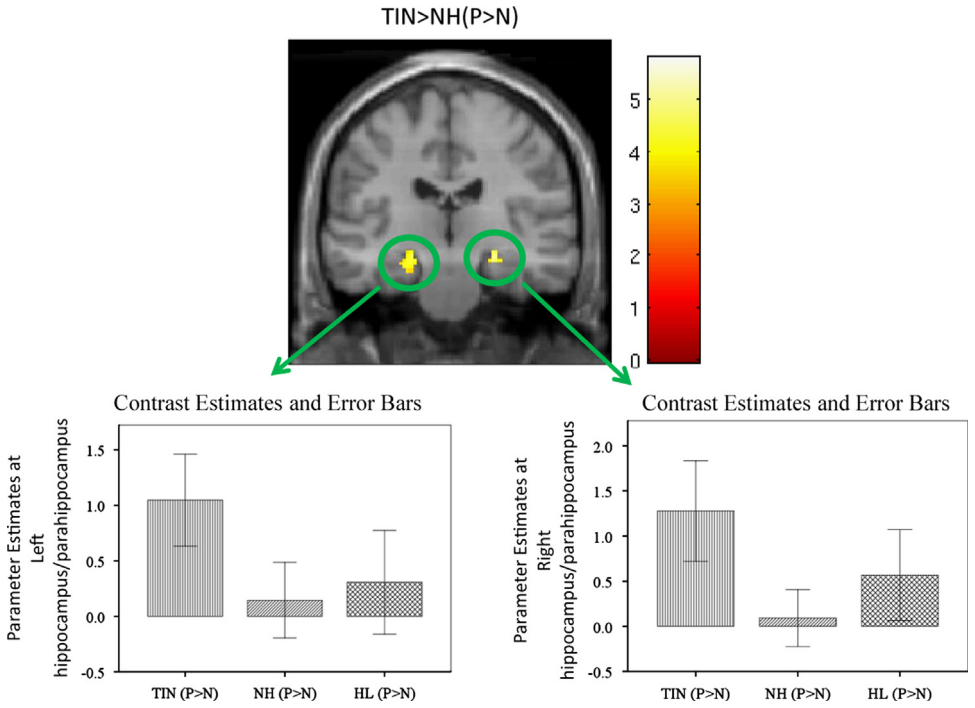


**Fig. 5 –** *Statistical parametric maps for post-hoc two sample t-tests.* **Post-hoc two-sample *t*-tests using ROI analysis that included insula and parahippocampus were used to determine group differences. Statistical tests detected increased hippocampus/parahippocampus response in the TIN group compared to controls for the P > N condition. For the local maximum located at bilateral hippocampus/parahippocampus for the TIN > NH (P > N) contrast, shown is increasing amplitude of effect for the three groups in the order: NH < HL < TIN. The maps are displayed at $p < 0.001$ uncorrected level for better visualization, but the clusters in the circles are corrected for multiple comparisons ($p < 0.05$ FWE).**

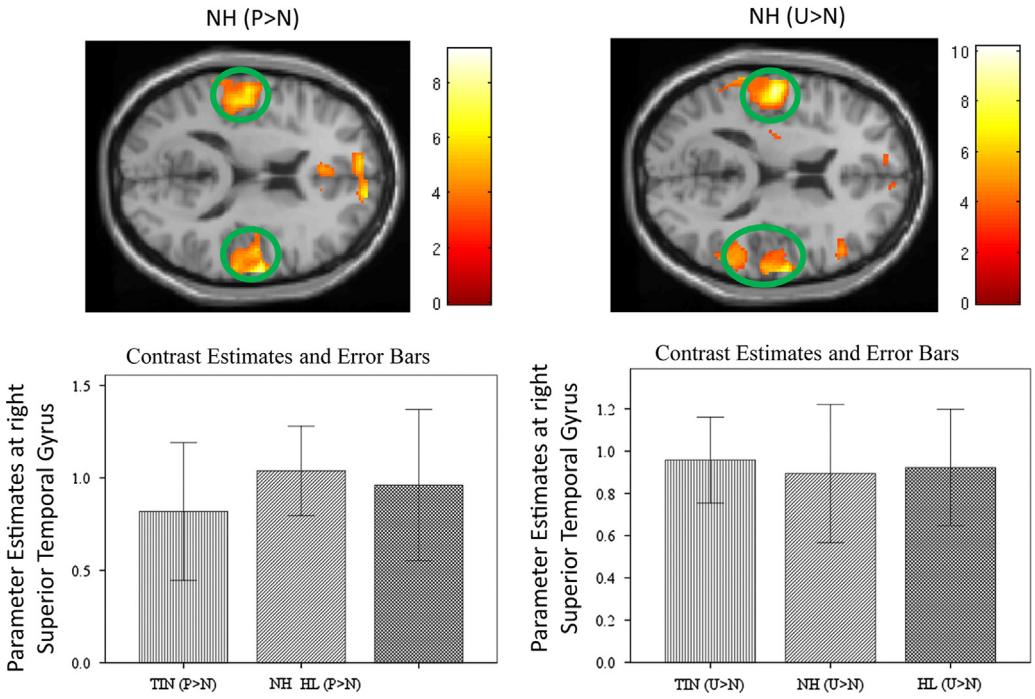


**Fig. 6 –** *Statistical parametric maps for the P > N and U > N contrasts.* **Within group, whole-brain voxelwise analysis revealed overlapping response from auditory regions in all three groups. Similar amplitude of effect was detected at the right superior temporal gyrus (BA22), for all three groups. Note that upon direct comparison with post-hoc two-sample *t*-tests using ROI analysis that included inferior colliculus, medial geniculate body, and primary auditory cortex (Brodmann areas 42, 41, 22), there were no significant differences between groups. The maps are displayed at $p < 0.001$ uncorrected level for better visualization, but the clusters in the circles are corrected for multiple comparisons ($p < 0.05$ FWE).**

**Table 4 – Local maxima for the effect of pleasant and unpleasant stimuli separately for each group.**

| Contrast | MNI coordinates X, Y, Z | Z score | Cluster ($mm^3$) | Gyrus (Brodmann area) |
|---|---|---|---|---|
| NH group (P > N) | 58, 2, −8 | 6.46 | 335 | R. middle temporal gyrus (BA 21) |
| | 64, −10, 12 | 5.98 | | R. transverse temporal gyrus(BA 42) |
| | 66, −26, 6 | 5.58 | | R. superior temporal gyrus (BA42) |
| | −54, −6,0 | 5.85 | 180 | L. superior temporal gyrus(BA 22) |
| | −50, −22, 16 | 5.43 | | L. insula |
| | −8,34,6 | 5.62 | 128 | L. anterior cingulate (BA 32) |
| | 4,38,4 | 5.59 | | R. anterior cingulate (BA 24) |
| | 18, −20, −24 | 5.42 | 28 | R. parahippocampal gyrus (BA 35) |
| | 4,58,20 | 5.08 | 17 | Medial frontal gyrus (BA 10) |
| NH group (U > N) | −54, −10, −2 | 6.82 | 931 | L. superior temporal gyrus (BA 21) |
| | −56, −2, −14 | 6.44 | | L. middle temporal gyrus (BA 21) |
| | 58,0, −8 | 6.78 | 1217 | R. middle temporal gyrus (BA 21) |
| | 62, −16, −2 | 6.66 | | R. superior temporal gyrus (BA 21) |
| | 64, −10,12 | 6.33 | | R. transverse temporal gyrus (BA42) |
| HL group (P > N) | −8,32,6 | 6.06 | 202 | Corpus callosum |
| | 14,42,6 | 5.96 | | R. anterior cingulate (32) |
| | 62, −8,12 | 5.95 | 240 | R. precentral gyrus (BA 43) |
| | 56, −22,12 | 5.87 | | R. transverse temporal gyrus (BA 41) |
| | 58,4, −10 | 5.78 | 78 | R. superior temporal gyrus (BA 21) |
| | 48,10, −6 | 5.49 | | R. superior temporal gyrus (BA 22) |
| | 56,8, −20 | 5.37 | | R. middle temporal gyrus (BA 21) |
| | 8,54,18 | 5.74 | 119 | Medial frontal gyrus (BA 9) |
| | 8,62,16 | 5.12 | | Medial frontal gyrus (BA 10) |
| | −26, −16, −4 | 5.69 | 24 | L. lentiform nucleus |
| | −52, −6,4 | 5.57 | 70 | L. superior temporal gyrus (BA 22) |
| | −54,2, −4 | 4.79 | | L. superior temporal gyrus (BA 22) |
| | −54, −14,52 | 5.41 | 15 | L. post central gyrus (BA 3) |
| | 10,8,4 | 5.36 | 23 | R. caudate |
| TIN group (P > N) | 58, −30,4 | 6.07 | 178 | R. precentral gyrus (BA 43) |
| | −8,52, −2 | 5.81 | 264 | R. superior temporal gyrus (BA 42) |
| | 4,40,6 | 5.43 | | R. superior temporal gyrus (BA 21) |
| | −8, −54,32 | 5.68 | 76 | L. inferior parietal lobule (BA 40) |
| | −40, −44,22 | 5.50 | 20 | L. superior temporal gyrus (BA 22) |
| | −56, −16,10 | 5.48 | 106 | L. superior temporal gyrus (BA 22) |
| | −48, −22,14 | 4.95 | | L. transverse temporal gyrus (BA 42) |
| | −56, −4,0 | 5.33 | 30 | |
| | −4,58,14 | 5.26 | 28 | |
| | 6, −62,28 | 5.20 | 11 | |
| | −54,12,2 | 5.12 | 13 | |
| TIN group (U > N) | −58, −16,12 | 7.15 | 52 | L. transverse temporal gyrus(BA 42) |
| | −48, −24,16 | 5.42 | 49 | L. insula |
| | 64, −10,10 | 5.19 | 71 | R. transverse temporal gyrus(BA 42) |
| | 56, −12,10 | 5.09 | | R. precentral gyrus (BA 43) |
| | 54, −30,4 | 5.05 | 13 | R. superior temporal gyrus (BA 22) |

Whole-brain analysis for both the P > N and U > N conditions was conducted for each group separately. Reported regions are listed in Montreal Neurological Institute (MNI) coordinates and in terms of Brodmann areas (before determining the Brodmann areas the MNI coordinates were converted to Talairach coordinates). Statistical threshold was set at $p < 0.05$ FWE corrected for multiple comparisons. L, left; R, right.

**Table 5 – Local maxima for the region-of-interest (ROI) analysis for within group contrasts.**

| Contrast | MNI coordinates X, Y, Z | Z score | Cluster ($mm^3$) | Gyrus (Brodmann area) |
|---|---|---|---|---|
| NH (P > N) | 30, −4, −18 | 3.30 | 6 | R. amygdala |
| NH (U > N) | 26, −4, −14 | 4.40 | 33 | R. amygdala |
| TIN (P > N) | | | | No suprathreshold voxels |
| TIN (U > N) | | | | No suprathreshold voxels |
| HL (P > N) | | | | No suprathreshold voxels |
| HL (U > N) | | | | No suprathreshold voxels |

Within group ROI analysis comprised of bilateral amygdala, defined bilateral, was conducted. Reported regions are listed in Montreal Neurological Institute (MNI) coordinates. Statistical threshold was set at $p < 0.001$ uncorrected. L, left; R, right.

stimuli was also evident in the TIN group; however, heightened amygdalar activity was not detected. Rather, increased activity was observed in the parahippocampus and insula. Therefore, the heightened parahippocampal and insular activity for the TIN group relative to controls may maintain the selective speed advantage of the affective stimuli. In support of this hypothesis, the HL group did not exhibit improved reaction time for the affective stimuli relative to neutral and increased activity in the parahippocampus and insula compared to the other groups was not detected. One explanation for the observed configuration may be that sensory deprivation may slow reaction times due to effortful listening as suggested by previous studies (Hicks and Tharpe, 2002; Tun et al., 2009). However, having tinnitus in addition to hearing loss may maintain a selective advantage for the processing speed of the affective stimuli. In sum, the present findings suggest that tinnitus may help maintain the selective speed advantage for affective stimuli through elevated engagement of the insula and parahippocampus.

### 3.2. *Amygdalar response detected only in the NH group*

Based on the literature, we hypothesized that the TIN group would show greater engagement of the amygdala when processing affective stimuli, especially unpleasant sounds, relative to controls (Henry et al., 2002; Jastreboff and Jastreboff, 2000; Mirz et al., 1999, 2000a, 2000b; Shargorodsky et al., 2010). The connections from the amygdala to the auditory cortex, which are important for judging the valence of a sound, may subserve such behavior (Kraus and Canlon, 2012; Kumar et al., 2012). Contrary to our hypothesis, our results did not show elevated response in the amygdala; rather, a decreasing trend of activation NH>HL>TIN was observed. One interpretation of the results may be that individuals with tinnitus re-route their emotional signaling pathway to avoid the amygdala and its connections to the auditory cortex (Kraus and Canlon, 2012; Kumar et al., 2012). In support of this notion, a decreasing trend of amygdala activity was observed when healthy adults were asked to decrease their emotional response to affective stimuli, and increased amygdala activity was observed when participants were asked to increase their emotional response to affective stimuli (Domes et al., 2010). Alternatively, a lack of increased response in the amygdala could be attributed to the subjects being well-adjusted to their tinnitus, as evidence by their rating of their tinnitus as slight-to-mildly bothersome on the THI scale. It is important to note that because amygdala response differences were not obtained when the groups were directly compared, our conclusions are tentative. Individuals with moderate to severe tinnitus may show increased engagement of the amygdala, as was initially hypothesized. Note that differences in activation of the NAc or the vmPFC for the TIN group compared to controls when processing either pleasant or unpleasant sounds relative to neutral were not observed. Our results demonstrate that individuals with mild tinnitus did not show increased engagement of the amygdala during emotional processing, contrary to our hypothesis.

### 3.3. *Heightened parahippocampal and insular response detected for the TIN group compared to controls, when processing pleasant sounds*

We expected the tinnitus group to show a heightened response in the insula and parahippocampus when processing the affective stimuli. This was confirmed when the groups were compared directly. For the TIN>NH (P>N) comparison increased bilateral parahippocampal and right insula activity was observed and for the TIN>HL (P>N) comparison increased left parahippocampal activity was detected. Past tinnitus research using resting state EEG and subjects with varying levels of tinnitus severity also found increased activity in the parahippocampus and insula related to tinnitus distress (Vanneste et al., 2010). The authors suggest that increased parahippocampal activity may be contributing to the conscious perception of tinnitus (Vanneste et al., 2010). They propose that in the non-tinnitus population, the parahippocampus may respond, but rapidly adapt to novel stimuli (Vanneste et al., 2010). In tinnitus, however, this mechanism of rapid adaption may be malfunctioning and thus the parahippocampus may be continually sending stored auditory information to cortical levels resulting in chronic tinnitus (Vanneste et al., 2010). Our study differs from the Vanneste et al. (2010) study in that all our participants had mild chronic tinnitus instead of a range to severe tinnitus, and the increased parahippocampal activity was specific to the P>N contrast; nevertheless, our results provide partial support for the Vanneste et al. (2010) group's findings.

In addition to elevated parahippocampal response, a heightened response in the insula for the TIN>NH (P>N) contrast was also observed. Increased resting state functional connectivity between the insula and frontal regions has been noted in subjects with severe tinnitus (Burton et al., 2012). Burton and colleagues propose that the increased connectivity is necessary for individuals with tinnitus to regulate their attentional resources in order to resolve the conflict between tinnitus and non-tinnitus sounds (Burton et al., 2012). In the Golm et al. (2013) fMRI study, increased activity in the insula, cingulate and frontal regions was observed in individuals with tinnitus compared to non-tinnitus controls when reading affective sentences. Our study differs from the Golm et al. (2013) study in that subjects in the latter study read sentences and a sub-group of participants had severe tinnitus and our participants listened to affective stimuli and did not have severe tinnitus. Nevertheless, our results partially support their findings in that increased activation for TIN>NH (P>N) comparison was shown to localize in the right insula. The researchers proposed that frontal, limbic and cingulate regions are positively correlated with tinnitus-related distress (Golm et al., 2013). An alternative view from the Haller et al. (2010) group suggested increased insular activity may be a compensatory mechanism. In their study, real time fMRI feedback was employed and the subjects were instructed to diminish their tinnitus (Haller et al., 2010). Although only two of the six subjects were able to successfully lessen their tinnitus, the researchers found decreased activation in the auditory cortex and increased activation in the insula when subjects attempted to diminish their tinnitus (Haller et al.,

2010). In our study, increased insular activity was observed in the TIN group, which had mild tinnitus, compared to NH controls suggesting that individuals with mild tinnitus relied on increased insular activity during emotional processing.

To parse out the effect of pleasant and unpleasant stimuli in greater detail, we examined the effect of each group for pleasant and unpleasant stimuli relative to neutral sounds separately. Our results suggest that pleasant sounds differentiate the tinnitus group from non-tinnitus controls to a greater degree than unpleasant stimuli. Increased response from parahippocampal and insular regions was found for the TIN > NH (P > N) comparison and parahippocampal regions for the TIN > HL (P > N) comparison. For the TIN > NH (U > N) and TIN > HL (U > N) comparisons, suprathreshold voxels were not detected. The TIN group may have a dampened neural response to unpleasant stimuli due a chronic unpleasant tinnitus precept. It is important to note that the subjects in our study had slightly-mildly bothersome tinnitus. Therefore, the TIN group may have employed successful coping strategies to ignore the tinnitus precept, and those strategies may also effect the processing of unpleasant sounds in general. Hence, it is important for future studies to include pleasant, unpleasant and neutral stimuli when investigating the limbic-auditory link in the tinnitus population.

### 3.4. *All three groups exhibited similar response patterns in auditory regions*

Increased activity in the temporal cortex to the affective sounds was observed in all three groups, as has been observed in earlier studies (Dietrich et al., 2007; Griffiths and Warren, 2002; Hunter et al., 2010). Upon direct comparison between groups, no differences were observed. We interpret the lack of difference in the central auditory pathway response in the three groups as that these pathways are involved with the processing of the acoustic information (i.e. the spectro-temporal content) rather than its valence. However, the present study did not examine functional connectivity between auditory regions which may be better able to elucidate potential connectivity differences between the groups (see for instance, Kumar et al., 2012).

A confounding factor the present study addressed was the effect of hearing loss, which has been shown to cause reorganization and decreased inhibitory input of the central auditory pathway (Brozoski et al., 2002, 2007; Dong et al., 2009; Kim et al., 1997; Syka, 2002; Szczepaniak and Moller, 1996), and may affect processing of affective sounds. Previous studies from our group (Husain et al., 2011a, 2011b) have shown some degree of similarity and differences between the tinnitus and hearing loss groups in functional response and anatomical connectivity of brain regions. In the current study, the NH and HL control groups showed increased response to both pleasant and unpleasant sounds in the frontal and auditory cortex. The behavioral results revealed that hearing loss may have contributed to the observed slower reaction time to both pleasant and unpleasant sounds compared to the normal hearing group. As noted in Table 6, there was no significant response to the HL > NH (U > N) and HL > NH (P > N) comparisons. This suggests that the TIN > NH and TIN > HL responses may primarily be due to tinnitus and not hearing loss. All participants with hearing loss reported that they could hear the sounds, and none of the TIN subjects reported that the scanner or stimulus sounds masked their tinnitus or that their tinnitus percept was affected by the scanning session.

Based on our findings, functional imaging may prove effective in objective detection of tinnitus presence and also lead to identification of brain regions that could be targeted with therapies. Functional imaging may provide valuable insight into the effectiveness of these treatment methods by evaluating brain response pre and post therapy, specifically those targeting the emotion processing region, such as

**Table 6 – Local maxima for the region-of-interest (ROI) analysis for inter-group contrasts.**

| Contrast | MNI coordinates X, Y, Z | Z score | Cluster $mm^3$ | Gyrus (Brodmann area) |
|---|---|---|---|---|
| TIN > NH (P > N) | 24, −20, −12 | 4.79 | 47 | R. parahippocampal gyrus (BA 28) |
| | 32, −12,22 | 4.74 | 11 | R. insula (BA 13) |
| | −20, −18, −14 | 4.44 | 60 | L. parahippocampal gyrus |
| TIN > NH (U > N) | | | | No suprathreshold voxels |
| NH > TIN (P > N) | | | | No suprathreshold voxels |
| NH > TIN (U > N) | | | | No suprathreshold voxels |
| TIN > HL (P > N) | −26, −24, −20 | 4.59 | 44 | L. parahippocampal gyrus |
| | 8,56,4 | 4.32 | 13 | Medial frontal gyrus (BA10) |
| TIN > HL (U > N) | | | | No suprathreshold voxels |
| HL > TIN (P > N) | | | | No suprathreshold voxels |
| HL > TIN (U > N) | 6,40, −8 | 4.71 | 26 | R. anterior cingulate (BA 32) |
| NH > HL (P > N) | | | | No suprathreshold voxels |
| NH > HL (U > N) | −14,34,26 | 4.98 | 20 | L. anterior cingulate (BA 32) |
| HL > NH (P > N) | | | | No suprathreshold voxels |
| HL > NH (U > N) | | | | No suprathreshold voxels |

Between group comparisons were computed with two sample *t*-tests using ROI analysis of amygdala, insula, parahippocampus, nucleus accumbens, ventral medial prefrontal cortex, inferior colliculus, medial geniculate body and primary auditory cortex (Brodmann areas 42, 41, 22), defined anatomically. Reported regions are listed in Montreal Neurological Institute (MNI) coordinates and in terms of Brodmann areas (before determining the Brodmann areas the MNI coordinates were converted to Talairach coordinates). Statistical threshold was set at $p < 0.05$ FWE corrected for multiple comparisons. L, left; R, right.

counseling or sound generation (Andersson, 2002; Jastreboff and Jastreboff, 2000). Future studies need to investigate whether the differences in emotional processing observed in the TIN group lead to perseveration of tinnitus or are a consequence of tinnitus. Functional connectivity differences in the emotional processing system of the TIN and control groups need to be explored for a more comprehensive characterization of the effect of tinnitus on the emotional processing network.

#### 3.4.1. *Caveats*

A no-stimulation condition was not included. Because of this, we are unable to comment on the nature of response to a type of sound relative to no sound/rest condition. This also precludes us from determining if there was elevated baseline for processing sounds in general in certain groups. For instance, an elevated baseline in the tinnitus group, relative to the control groups, would suggest that sound processing in general is engaging the limbic system in a relatively-neutral context of MRI scanning (Gu et al., 2010; Melcher et al., 2009; Seydell-Greenwald et al., 2012). Due to experimental design constraints, we used sounds with high arousal scores. Future studies employing an equal number of high and low arousing sounds may better explain the effects of arousal (different from valence) on emotional processing. Another limitation of the study is that we included adults who had tinnitus for different lengths of time and this may have affected their neural response patterns. However, to partially control for this potential source of variance, we excluded participants with recent onset of tinnitus (defined to be less than one year in duration). Despite these limitations, the present study serves as a baseline for future studies of individuals with severe and bothersome tinnitus.

## 4. Conclusion

In conclusion, we found evidence for behavioral and emotional processing differences between tinnitus and control groups, suggesting an interaction between tinnitus and the limbic system. Behaviorally, individuals with tinnitus maintained a similar reaction time profile with that of individuals with normal hearing, thus indicating that tinnitus may preserve the rapid processing of emotional stimuli in the presence of hearing loss. Contrary to our expectation, increased amygdala activity was not detected for the TIN group compared to controls; rather, a decreasing trend of activation NH > HL > TIN was observed. The TIN group had slightly-mildly bothersome tinnitus which may explain the relatively low amygdalar response in the TIN group compared to controls. The increased response observed in the parahippocampus may indicate a lack of habituation to the novel precept, resulting in tinnitus persistence. Increased insular activity may be an indication of compensatory mechanisms to manage tinnitus distress. The similar response from auditory regions in all three groups to the affective sounds may reflect these pathways processing acoustic information rather than valence. We intend to conduct other task- and rest-based functional connectivity studies, to isolate the contributions of the different nodes of the emotion processing network and their connections.

## 5. Experimental procedures

### 5.1. *Subjects*

Subjects from three groups were recruited from the Urbana-Champaign area and given written informed consent. Participants were scanned under the UIUC IRB 10144 protocol and were suitably compensated. There were three groups: bilateral hearing loss with tinnitus (TIN), bilateral hearing loss without tinnitus (HL) and normal hearing without tinnitus (NH). The tinnitus volunteers consisted of 13 participants with bilaterally symmetric mild to moderately-severe hearing loss and chronic subjective tinnitus. Tinnitus severity was assessed using the tinnitus handicap inventory (THI) (Newman et al., 1998). Using medical history questionnaires and auditory testing, HL and TIN subjects who exhibited asymmetric high frequency hearing loss and/or lateralized tinnitus were excluded from the study. Asymmetric hearing loss was defined as follows: if the right and left ear differed more than 15 dB HL at one or more frequency or if at two consecutive frequencies the right and left ear varied 10 dB HL. TIN participants with slightly-to-mildly bothersome tinnitus (defined as THI score less than 30) were included in the study; one TIN subject was excluded for an outlier THI score of 46. The HL ($n=12$) participants served as a hearing loss control group. HL subjects were selected based on their audiological assessment, described in the next section, to serve as controls for the TIN group's hearing loss. Finally, the NH participants ($n=12$) served as an age matched normal hearing control group. The NH group had bilateral normal hearing ranges and no tinnitus. Subjects from all groups were predominantly right handed (TIN: 12 right handed, 1 left handed; HL: 11 right handed, 1 left handed; NH: 12 right handed, 0 left handed). All subjects, regardless of group, scored in the minimal depression range for the Beck Depression Inventory (BDI-II) and minimal anxiety range for the Beck Anxiety Inventory (BAI) (Beck and Steer, 1984; Steer et al., 1993, 1999). In addition to the present study, the NH and HL subjects were included in a separate investigation of neural differences between individuals with and without hearing loss. See Table 1 for subject demographics.

### 5.2. *Audiometric evaluation*

All subjects underwent a full audiometric evaluation, conducted inside a sound-attenuating booth. Pure tone testing, word recognition testing, and bone conduction testing were done for each subject. In order to eliminate the possibility of any associated peripheral hearing pathology besides tinnitus, distortion product otoacoustic emissions and tympanometry measurements were conducted. All participants in the NH group had pure-tone thresholds of 25 dB HL or lower for all test frequencies (0.25, 0.5, 1, 2, 4, 6, 8 kHz). For test frequencies.25 kHz – 2 kHz, subjects in the TIN and HL group had a pure-tone threshold of 30 dB HL or lower. At 1000 Hz, two HL subjects had a minor elevated pure-tone threshold of 35 dB

HL. The HL and TIN subjects had hearing loss between mild and moderately-severe for frequencies 4–8 kHz with pure-tone thresholds ranging from 30 to 70 dB HL. See Fig. 1 for average audiogram.

### 5.3. Stimuli and task

Sound stimuli were obtained from the IADS database (Bradley and Lang, 2007), which has normative scores on a 9-point scale for valence (9 very pleasant, 1 very unpleasant) and arousal (9 very arousing, 1 not at all arousing). Sounds were rated to be pleasant (valence: 6.83±0.54, arousal: 6.46±0.56), unpleasant (valence: 2.78±0.58, arousal: 6.9±0.31) and neutral (valence: 4.81±0.43, arousal: 4.85±0.57), while controlling for arousal for the pleasant and unpleasant sounds. The sounds were broad-band with varying peak and average amplitudes (for details see the IADS technical report: Bradley and Lang, 2007). No attempt was made to alter the sounds to compensate for the subjects' high-frequency hearing loss, in order to maintain the ecological validity of the participants' listening experience. Sounds were presented at a maximum comfort level during "relatively-quiet" time periods of clustered fMRI acquisition. Prior to data acquisition, participants were trained on the task with a set of sounds from the same database, which were not used in the final experiment, and written and verbal instructions were provided. Sounds and instructions were provided to the participants using Presentation 14.7 software (http://www.neurobs.com) on a Windows XP PC in the fMRI control room through pneumonic headphones (Resonance Technology, Inc., Northridge, CA). Subjects were presented with 90 affective auditory stimuli (30 pleasant, 30 unpleasant, 30 neutral), each 6 s in duration. Participants denied having any difficulty hearing the sounds. The ratings of sounds, as pleasant, unpleasant or neutral, were obtained by button presses. Subjects were instructed to respond as soon as they felt confident in their rating. In the fMRI data analysis, trials were coded depending on the individual subject's ratings of the sounds, rather than the rating provided by IADS.

### 5.4. Data acquisition

Ninety EPI image-volumes were collected from each subject using a 3Tesla Siemens Magnetom Allegra head-only scanner. Sparse sampling or clustered echo-planar imaging (EPI) acquisition was used in the present study (Husain et al., 2011b). This method prevents the loud noise of the radio-frequency gradients of the scanner from masking the subject's tinnitus or interfering with the perception of the sounds, thereby boosting response in the auditory pathways (Gaab et al., 2003; Hall et al., 1999; Zaehle et al., 2004). This was particularly important in the current experiment because several subjects had hearing loss and/or tinnitus. During clustered acquisition, one image volume (2 s) was collected following the presentation of the auditory stimulus, instead of continuous image acquisition. The 6 s sound stimulus was played during a 7 s interval of reduced scanner noise and the repetition time was 9 s. A custom MATLAB (http://www.mathworks.com/products/matlab/) toolbox previously used in the Dolcos and McCarthy (2006) study was employed prior to data collection to identify peak neural response of different brain regions that constitute the limbic system (Dolcos and McCarthy, 2006). The toolbox allows selective averaging of the fMRI response on a timepoint-by-timepoint basis without any assumptions of the HDR function, and is available at Duke University's Brain Imaging and Analysis Center (http://www.biac.duke.edu). The results obtained from this peak detection were used to fine-tune the timing of stimulus presentation relative to image acquisition.

A series of two anatomical and one functional image were acquired. First, 32 low-resolution T2-weighted structural transversal slices were acquired for each volume (TR=7260 ms; TE=98 ms; 4.0 mm slice thickness; $0.9 \times 0.9 \times 4.0\ mm^3$ voxel size matrix size (per slice); $256 \times 256$; flip angle, 150°). A total of 160 high resolution magnetization-prepared rapid-acquisition with gradient echo (MPRAGE) sagittal slices were collected for each volume (TR, 2300 ms; TE, 2.83 ms; 1.2 mm in thickness; $1.0 \times 1.0 \times 1.2\ mm^3$ voxel size; matrix size (per slice), $256 \times 256$; flip angle, 9°). The functional image acquisition parameters were: slice thickness, 4 mm; inter-slice gap, 0.4 mm; 32 axial or transverse slices, distance factor, 10%; voxel size, $3.4 \times 3.4 \times 4.0\ mm^3$; field of view (FoV) read, 220 mm; TR, 9000 ms with 2000 ms acquisition time; TE, 30 ms; matrix size (per slice), $64 \times 64$; flip angle, 90°.

### 5.5. Data analysis

#### 5.5.1. Behavior

Behavioral data were analyzed with SPSS ver. 20 software (statistical package for social sciences, IBM, http://www-01.ibm.com/software/analytics/spss/). Separate two-way ANOVAs were conducted for the following depen dent variables (1) reaction times, (2) ratings obtained for the three types of sounds. The independent fixed factors used in the general linear model were: group (TIN, NH, HL) and stimulus condition (P, pleasant; U, unpleasant; N, neutral). For all behavioral data the significance was set at $p<0.05$.

#### 5.5.2. fMRI

fMRI data were processed and analyzed using SPM8 (Statistical Parametric Mapping, Welcome Trust Center for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) software. Rea lignment of the images was done using a rigid-body transforma tion to neutralize head motion. A two-step SPM coregistration and normalization process was employed. The low resolution Axial (Ax) T2 image was first co-registered with the mean EPI image produced during realignment. Next, the high resolution MPRAGE image was registered with the AxT2 image, in order to be in the same alignment. The MPRAGE image was then spatially normalized to match a standard T1 MNI template. The normalization parameters obtained during the process were applied to the functional EPI images, thus resulting in normalized functional images in standard MNI space. The normalized images were smoothed with a Gaussian kernel of $8 \times 8 \times 8$ FWHM.

First, to obtain P>N and U>N contrast images first level fixed effects analysis was performed on each subject's smoothed images. To model BOLD signal, canonical hemodynamic response function within SPM, with an interscan interval of 9 s, microtime resolution of 18 and microtime

onset of 15 to model a 2 s event (occurring 5–7 s prior to volume acquisition) was used. Motion regressors were included in the model to account for motion-related artifact and a high-pass filter set at 550 s was incorporated. The P>N and U>N contrast images generated from each subject during first level fixed-effects analysis were used in a flexible factorial analysis. Flexible factorial design was employed at the second level because it allows flexibility in choosing main effects and interactions in the general linear model. The design included three factors: group assumed to be independent and have unequal variance, subject assumed to be independent and have equal variance, and condition assumed to be dependent and have equal variance. Within the flexible factorial model, post-hoc two-sample *t*-tests were conducted to identify distinctions of the TIN group with respect to the control groups for specific stimulus types. Centered on our *a priori* hypothesis concerning the involvement of auditory and limbic regions in affective sound processing targeted ROI analysis was conducted. The Wake Forest University pickatlas toolbox (http://www.fmri.wfubmc.edu) within SPM8 was used to con duct additional ROI analysis. An anatomically defined mask was generated using regions within the limbic and auditory systems consisting of amygdala, insula, parahippocampus, nucleus accumbens, ventral medial prefrontal cortex, inferior colliculus, medial geniculate body and primary auditory cortex (Brodmann areas 42, 41, 22) and used for between group comparisons. The amygdala is difficult to detect using fMRI due to its small size and location (Irwin et al., 2012). Therefore, a separate ROI mask comprised solely of the amygdala was used for within group analysis with a threshold of $p<0.001$. Small volume correction was implemented for each ROI analysis for the effect of pleasant or unpleasant stimuli separately for each group and for inter-group contrasts. Statistical significance was set at $p<0.05$ FWE corrected for multiple comparisons using random field theory at the voxel level for fMRI data analysis.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.brainres.2014.04.024.

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